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STERILIZATION OF NATURALLY CONTAMINATED METAL SURFACES
WITH DRY HEAT

(NASA CR-52899)

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Protection Branch Report of Test No. 8-64

Sterilization of Naturally Contaminated Metal Surfaces
With Dry Heat

Previous studies in these Laboratories have shown electronic components and potting compounds to be frequently contaminated internally, thus requiring some sterilization treatment if they are to be used in the construction of an interplanetary spacecraft. When heat was first suggested as a technique for sterilizing a spacecraft or its components it was apparent that only dry heat could be considered since moisture can not penetrate sealed materials. Dry heat, however, is much less effective than moist heat. The usual practice is to expose articles for something like four hours at 160°C as compared to about 20 minutes at 125°C for moist heat. At lower temperatures dry heat sterilization exposure times are of such length that they were considered impractical for routine use in laboratories and consequently few sterilization studies have been conducted along this line. Not all electronic components can withstand 160°C , however, so it then became important to determine the minimum temperature for sterilizing under dry conditions, even if it necessitates quite long exposure times.

One recommended value for dry heat sterilization reported in the literature ^{1/} was for overnight exposure, or longer, at 121°C . There is little point in testing temperatures much lower than this since, even in the presence of moisture some thermophilic bacteria can survive temperatures exceeding 100°C . On the basis of the little information appearing in the literature and a few laboratory tests ^{2/} 125°C for 24 hours was recommended as a tentative set of conditions for sterilization and it was suggested that immediate effort be undertaken to gather further experimental data on this subject. Accordingly, in the spring of 1961 the National Aeronautics Space Administration contracted with the Wilmot Castle Company to collect such information. In the conduct of the Wilmot Castle studies ^{3/}, they found cases where heavily contaminated soils were not sterile after exposing to 125°C for 24 hours. Accordingly, higher temperatures have been proposed, among them 135°C for 24 hours.

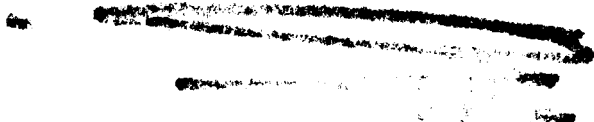
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The time required to sterilize any object under a given set of conditions will depend on the number of viable microorganisms which must be destroyed, their natural resistance, and the protection afforded them by extraneous material. In order to obtain a reasonable estimate on the first point, a recent study ^{4/} was undertaken by this Laboratory to determine the level of microbial contamination that accumulates on surfaces from aerial fallout and also from handling. In essence this study revealed that the number of microorganisms that would collect on a spacecraft during assembly is several orders of magnitude less than estimated previously ^{4/}. About 100 organisms or less per square inch were collected after one month exposure and the number did not continue to increase when the exposure was extended to three months. A second study is now in progress to obtain similar information under clean room conditions. These studies were run only to determine the extent to which surfaces become contaminated and no attempt was made to sterilize the natural contamination by heat or other means.

No effort was made to determine the natural resistance of the microorganisms, the second point mentioned above. The amount of extraneous matter on a spacecraft is undoubtedly very small so that while soil does afford some protection to microorganisms from the lethal effect of heat it is probably not a desirable media in which to conduct tests for the purpose of selecting a realistic time-temperature combination for spacecraft sterilization. Since all previous heat sterilization studies were conducted on artificially contaminated surfaces Mr. L.B. Hall of NASA headquarters requested a brief test to determine whether the naturally contaminated surface is readily sterilizable by heating at 135° C for 24 hours. The results of this study are reported herein.

MATERIALS AND METHODS

Two replicate tests were conducted during summer by placing sterile stainless steel strips (1 x 2 inches) in a horizontal position on a clean decontaminated shelf. The shelf was about five feet above the floor in a small room in a non-airconditioned chemical-biological laboratory building. Thirteen stainless steel strips used for each test were exposed to room air for 33 days. The replicate tests were initiated one week apart, so for 26 days the exposures coincided. From each test, ten stainless steel strips were subjected to dry heat at 135° C while the remaining three strips were assayed to determine the number of viable microorganisms present on the unheated samples. Five strips from each test were heated at 135° C



for 12 hours and five at 135° C for 24 hours. Immediately following the heating period, each strip was placed in a separate fluid thioglycollate medium blank and then incubated at 37° C for seven days before checking for sterility. The unheated strips were placed in separate 50 ml sterile distilled water blanks and shaken. Forty-five ml of the unheated sample were assayed by the pour plate method for viable microorganisms; 30 ml of this were used for the numeration of aerobes and 15 ml for the numeration of anaerobes. The aerobes were cultured in tryptose agar at 25° C for 48 hours and the anaerobes were cultured in thioglycollate agar at 37° C for 48 hours.

RESULTS AND DISCUSSION

Table I shows that almost 1000 microorganisms were recovered from a stainless steel strip (approximately 500/sq in) after exposure to room air for 33 days. None of the stainless steel strips exposed to 135° C for 12 hours or for 24 hours contained any viable microorganisms as evidenced by the lack of microbial growth after seven days incubation in fluid thioglycollate medium. After the seven day incubation period broth samples in which the strips had been placed all supported bacterial growth when about 100 spores of Bacillus subtilis var niger were deliberately introduced.

The number and type of viable microorganisms on a surface at any specific time varies considerably. In these tests a somewhat higher number of organisms was recovered from the surfaces than in the previously reported work ^{4/}. Almost all of the microorganisms recovered were aerobes, with spore-forming bacteria the most predominant type, judging from visual observations of colonial formation.

Although one cannot say categorically that based on the results reported herein, a spacecraft can be sterilized by heating to 135° C for 24 hours, the data greatly support the choice of this time-temperature combination as adequate, particularly if care is taken to keep the spacecraft clean during assembly.

References

1. Perkins, J.J. 1957. Bacteriological and Surgical Sterilization by Heat. Ch. 31. In Antiseptics, Disinfectants, Fungicides and Sterilization by G.F. Reddish. Lea and Febiger, Philadelphia, Pennsylvania.
2. Protection Branch Report of Test No. 22-60: Effect of Dry Heat Upon Dry Bacterial Spores. Physical Defense Division, Fort Detrick, Maryland, 14 April 1960.
3. NASA Contract NASr-31 and NASw-550.
4. Protection Branch Report of Test No. 1-64: Microbial Contamination Obtained on Surfaces Exposed to Room Air or Touched by the Human Hand. Physical Defense Division, Fort Detrick, Maryland, 22 July 1963.

Table 1.

Recovery of Microorganisms From Unheated and Heated Stainless Steel Strips After Exposure to Room Air for 33 Days

Unheated

	<u>Microorganisms/Strip (2 sq in)</u>			
<u>Test</u>	<u>Molds</u>	<u>Aerobes</u>		<u>Anaerobes</u>
		<u>Bacteria</u>	<u>Total</u>	
1	170	820	990	0
2	540	410	950	3

Heated at 135° C

<u>Test</u>	<u>12 Hours</u>	<u>24 Hours</u>
1	0	0
2	0	0

Note: Each entry is an average of three to five determinations.